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(54) 【発明の名称】 二次元電気泳動用プレキャストゲルシステム

(57) 【特許請求の範囲】

【請求項1】 細長い片の形の第一のゲル及びスラブの形の第二のゲルよりなり、前記の第一及び第二のゲルは単一のゲル支持手段上に保持され、さらに室温で固定でありしかも約25℃と約75℃との間の融点を有する非導電性の材料により互いにその上で離れているプレキャスト二次元電気泳動ゲルシステム。

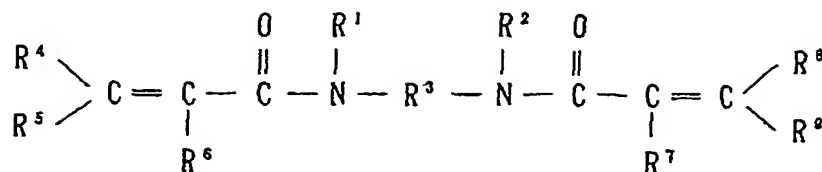
【請求項2】 細長い片の形の第一のゲル及びスラブの形の第二のゲルよりなり、前記の第一及び第二のゲルは、単一のゲル支持手段上に保持され、さらに室温で固定でありしかも非水性の溶媒中で室温で可溶な非導電性の材料により、互いにその上で離れているプレキャスト二次元電気泳動ゲルシステム。

【請求項3】 該ゲル支持手段が一組の実質的に平行な支

持板よりなる請求項1または2記載のプレキャスト二次元電気泳動ゲルシステム。

【請求項4】 該ゲル支持手段が、一組の実質的に平行な支持板よりなり、そして前記の第一及び第二のゲルが、該支持板の間に配置されて、前記の第一及び第二のゲルを乱すことなく該支持板から前記の除去可能な電氣的に絶縁されている層を除去せしめる開放通路を残す請求項1または2記載のプレキャスト二次元電気泳動ゲルシステム。

【請求項5】 前記の第一及び第二のゲルがポリアクリルアミドゲルであり、前記の第一のゲルが蛋白可溶化量の尿素を含み、前記の第二のゲルが蛋白可溶化量のナトリウムドデシルサルフェートを含み、そして前記の第一のゲル中の該ポリアクリルアミドが式



(式中、R¹、R²及びR³は以下の様に規定され、

即ち

R¹及びR²は独立してC₁~C₅アルキルでありそしてR³は、C₁~C₈アルキレンであるか；又は

R¹及びR²は結合してC₁~C₈アルキレンを形成しそしてR³はC₁~C₈アルキレンであるか；又は

R¹はR³に結合して3~10個の炭素原子の飽和ヒドロカルビル基を形成しそれはR¹及びR³が結合しているN原子と一緒にN含有環を形成し、そしてR²はC₁~C₅アルキルであるか；又は

R¹及びR²はR³に結合して7~15個の炭素原子の飽和ヒドロカルビル基を形成しそれはN原子と一緒に2個のN含有環を形成し；そして

R⁴、R⁵、R⁶、R⁷、R⁸及びR⁹は独立してH及びC₁~C₅アルキルよりなる群から選ばれる)

を有する化合物と橋かけ結合剤と橋かけ結合している請求項1または2記載のプレキャスト二次元電気泳動ゲルシステム。

【請求項6】二次元電気泳動ゲルを注型する方法において、該方法が、

(a) 一組の支持板の間に第一のゲルを形成し、前記の第一のゲルの露出した縁に沿って該支持板の間に空いたスペースを残し；

(b) 非導電性の材料を前記の空いたスペースに置いて該スラブの前記の露出した縁に隣接する第一の層を形成し、該非導電性材料が室温で固体でありしかも約25℃と約75℃との間の融点を有するものであり；

(c) ゲル形成液体を前記の空いたスペースに置いて、前記の第一の層に隣接する細長い第二の層を形成し、該物質及び該ゲル形成液体が実質的に不混和性であり；

(d) 前記の第二の層を細長いゲルに形成し；そして

(e) 該導電性材料を該融点又はそれより高い温度に加熱し、そして該支持板から液体の状態の該物質を取り出すことによりなる方法。

【請求項7】サンプルを二次元電気泳動により成分に分離する方法において、該方法が

(a) 細長い形状を有する細長い片の形の第一のゲル及びスラブの形の第二のゲルよりなる二次元電気泳動ゲルの配置を提供し、前記の第一及び第二のゲルが単一のゲル支持手段上に保持され、そして前記の第一及び第二のゲルが室温で固定でありしかも約25℃と約75℃との間の融点を有する材料の介在領域により分離されており；

(b) 該サンプルを前記の第一のゲル上的一端に載せ；

(c) 前記の細長い形状に平行な方向に前記の第一のゲ

ルの間に電場をかけ、該サンプルの該成分の電気泳動分離をそこで行なってゾーンとし；

(d) 該非導電性材料を該融点より高く加熱し；

(e) 液体の状態にある間に該非導電性材料を取り出し；

(f) 前記の第一のゲルを前記の第二のゲルに向かって移動して前記の第一及び第二のゲルを直接接触させ；そして

(g) 前記の細長い形状を横切る方向に前記の第一及び第二のゲルの両方の間に電場をかけて、前記の第二のゲルの該ゾーンの電気泳動分離を行なう

ことよりなる方法。

【請求項8】サンプルを二次元電気泳動により成分に分離する方法において、該方法が

(a) 細長い形状を有する細長い片の形の第一のゲル及びスラブの形の第二のゲルよりなる二次元電気泳動ゲルの配置を提供し、前記の第一及び第二のゲルが単一のゲル支持手段上に保持され、そして前記の第一及び第二のゲルが室温で固体でありしかも約25℃と約75℃との間の融点を有する材料の介在領域により分離されており；

(b) 該サンプルを前記の第一のゲル上的一端に載せ；

(c) 前記の細長い形状に平行な方向に前記の第一のゲルの間に電場をかけ、該サンプルの該成分の電気泳動分離をそこで行なってゾーンとし；

(d) 該非導電性材料を該融点より高く加熱し；

(e) 該非導電性材料を液体の状態のある間に導電性ゲル形成液体により置換し；

(f) 該ゲル形成液体を導電性ゲルに形成し；そして

(g) 前記の細長い形状を横切る方向に前記の第一及び第二のゲルの両方の間に電場をかけて、前記の第二のゲルの該ゾーンの電気泳動分離を行なう

ことよりなる方法。

【発明の詳細な説明】

【産業上の利用分野】

本発明は二次元ゲル電気泳動に関し、特に二次元のゲル分離に用いられるゲルの製法及び使用法に関する。

【従来の技術及び発明が解決しようとする課題】

二次元電気泳動は、複雑な蛋白混合物を分離するのに広く用いられている。分離を、連続して2種の異なる組の性質に基づいて生じさせることにより、二次元電気泳動は単一段階の分離で得られるそれよりも非常に高い分解能力をもたらす。

技術を用いて種々のやり方で分離パラメーターを組合せる。例えば電荷に基づく分離が第一の段階で行なわれ、次に分子量に基づく分離が第二の段階で行なわれ

る。同様に、一つのゲル濃度における分離の次に、同じゲルの他の濃度における分離がなされる。他の例として、二段階使用によるpHの段階的变化、均一ゲル次に多孔性の勾配ゲル、2種の異なる蛋白溶解剤又は同じ溶解剤の2種の濃度の使用、一つの段階における不連続なバッファー系そして他の段階における連続なバッファー系、そして均一又は多孔性の勾配電気泳動をとともう等電点電気泳動。このような技術は、血清又は細胞の蛋白、細菌性蛋白、非ヒストンクロマチン蛋白、リボソーム蛋白、リボ核蛋白及びリボソーム蛋白の混合物、核酸及び同様な物質の分離を行なわせしめる。

二次元系の基本的な方法は、細長い又は棒状のゲル例えば5.0mmのオーダーの直径を有するものの一次元分離で始まり、溶質又は蛋白が棒の長さに沿って位置するゾーンの中に分布するまでゲルの軸に沿って泳動及び分離が生ずる。次にスラブゲルの一つの縁に沿って棒を配置して、棒のゲルの軸を横切る方向に各ゾーンからスラブゲルに溶質の第二の次元の泳動を行なう。

このタイプのやり方で遭遇する困難は、第二の次元の分離を行なうために第一の次元の分離が生じた後の棒状のゲルの移動に関するものである。第一の次元の分離は、棒のゲルが最初注型された管内で一般に生ずる。第一の次元の分離が完了すると、溶質ゾーンを有するゲルの棒は、物理的な手段例えば抽出により管から取り出され、次にスラブゲルの露出した縁に沿って置かれる。これらの操作は、微妙な取り扱いを必要とし、たとえ非常に注意を働かせても、ゲルを損ないそして溶質ゾーンの歪みや乱れを生ずる危険が残っている。一度抽出されると、棒のゲルは、スラブゲルと適切に配列され、そして電気的な連続性及びゲル間の妨害されない溶質の泳動の両方の目的のために十分に接触される。これらは、誤りの他の源であり再現性を損なう。処理の困難さ並びに不正確さ及び再現性の損失の可能性に加えて、棒のゲルの取り扱い及び配置に要する時間は、操作者の能率を低下させ、どんな致命的な誤りも取り返しのつかない時間とデータの損失を招くことになる。ある人々は、第一の次元の分離にゲルの片を用いるが、同様な困難、誤り及び再現性の損失に遭遇する。

〔課題を解決するための手段〕

本発明は、プレキャスト (precast) 二次元ゲルシステムに関し、その第一及び第二の次元のゲルは、分離の両方の段階が生ずる単一のゲル支持体上に組み合わせられている。支持体は、単一の組の板よりなる囲みであり、適切なスペーサーを有して板の間に空間を設けゲルの厚さを規定する。第一及び第二の次元のゲルは、ともに注型され板の間に保持される。第二の次元のゲルは、板間の空間の一部を占めるスラブであり、一方第一の次元はスラブに平行に残りの空間に位置する片であり、ゲルを分ける除去可能な電気的に絶縁する層を有する。又、支持体は、プラスチックのシートよりなるゲル・バックキ

ング、ゲル・ボンド又は他の支持材料であり、その上に第一及び第二の次元のゲルがともに注型され、そしてそれらの間に絶縁材料の層又は空気のスぺースである絶縁層を有する。

第一の次元の分離は、従って片状のゲルの一端にサンプルを載せ、2種のゲルを互いに電気的に絶縁する一方、長さ方向に片状ゲルの間に電場をかけ、次に電気的に絶縁する層を取出し、2種のゲルをそれらの完全な長さに沿って電気的接触をし、次に第一のそれを横切る方向に両者の間に電場をかけることにより行なわれる。電気的に絶縁する層は、固体、液体又は気体でも良く、そして第二の次元の分離における2種のゲルの間の電気的接触は、直接接触により又は導電性の層の挿入によりの何れかで達成される。本発明は、分離の2種の段階の間で一つの支持囲みから他のものに棒又は片状ゲルを移動する必要をなくし、それによりゲルの損失及びゾーンの歪みの危険を低下させ、そして不正確さ及び定誤差の発生を減少させることになる。

本発明の他の目的、利点及び特長は、下記の記述から明らかであろう。

第1図は、本発明によるゲルを注型し保持するのに用いられるのに適したゲルの囲みの分解透視図である。

第2図は、第二の次元のゲルの注型直後の一枚の支持板を除いた、第1図のゲルの囲いで製造された二次元ゲルシステムの正面図である。

第3図は、分離の第一の段階中の再び板の一枚を除いた第2図のゲルシステムを示す。

第4a及に4b図は、第二の段階の開始の条件の第2及び3図のゲルシステムを示す。

本発明によれば、片の形の細長いゲルが、従来の技術の棒状のゲル又は分離したゲル片の代わりに第一の次元の分離に用いられる。片状ゲルは、スラブゲルの上の浅い層として、スラブゲルと同じやり方で支持板の間に注型される。これは、好ましくはスラブゲルが形成された後になされる。二つの間の絶縁層は、固体、液体又は気体でも良く、片ゲルの注型中に存在するか（この場合、それは又2種のゲル間の流体の接触を防ぐバリアーとして働く）又は片状ゲルが注型されたならばバリアー層の代わりとなるの何れかである。

種々の層及びゲルの囲いが、一度第一の次元の分離が完了したとき絶縁層が除かれるようなやり方で、成形されそしてそのような大きさとする。これは、ゲルの囲いの外部と絶縁層の領域とを連絡する開放した通路を設けることにより一般になされる。通路は、好ましくは片状ゲルの一端又は両端の回りのクリアランスの形であり、そこでは絶縁層は排除されることにより外方に押されるか又は取り去られるの何れかである。

添付の図面は、本発明の一つの説明的な態様の詳しい図を提供する。

第1図は、電気泳動の両方の段階のためのゲルの注型

及び使用に用いられるのに適した、分解した形のゲルの囲い11を示す。囲みの構成部品は、2枚のガラス板、一組の側面スペーサー14、15及び上部スペーサー16を含む。側面スペーサー14、15は、従来のスラブゲルの囲みにおけるように、ガラス板12、13の間に空間を設け、それにより板間に注型されるゲルの厚さを決める。図示された側面スペーサーは、均一の厚さを有し、同様に均一の厚さのゲルをもたらす。又、スペーサーの長さに沿って変化する厚さを有する側面スペーサー例えば楔形のスペーサーも、垂直方向にゲルの厚さを変える目的で、用いてもよい。

側面スペーサー14、15及びガラス板12、13は、各側面に沿ってクランプ（図示せず）によりサンドウィッチのタイプの配置で互いに保持される。ゲル溶液は、ガラス板と側面スペーサーとの間の空間17に注がれ、従来の方法に従って、ゲルに固化せしめられ、囲みは注型用スタンドに垂直にマウントされる（図示せず）。適当なクランプ、注型用スタンド及びゲルを形成するのに要求される他の器具は、従来広く用いられているものであり、入手可能である。

側面スペーサー14、15は、クランプにより所定位置に固定される。しかし、上部スペーサー16は、除去可能であり、片状ゲルの注型中に用いられる。その使用は、第2図に関してさらに明瞭に示される。

第2図において、第1図の前方のガラス板12は、明瞭さのために除かれる。図に残っているのは、後方のガラス板13、2個の側面スペーサー14、15及び除去可能な上部スペーサー16である。

第1図に戻って、側面スペーサー14、15は、それぞれ内部の空間17に向う肩部21、22を備えることに注意すべきである。この態様では、これらの肩部は、それからスラブゲル23（第2図）が形成される溶液を注ぐための液体のレベルのしるしをつける。スラブゲルの上部の縁24は、従ってこれらの肩部と同一平面にある。スラブゲルを注型するのに、ゲルの囲み11は上部スペーサー16を含むことなく組み立てられ、ゲル溶液は肩部12、22のレベルに注がれ固化される。

一度スラブゲル23が十分に形成されると、保護層25がその上に置かれ、その上部の縁24をシールする。この保護層は、下記に説明されるように、形及び組成が変化できる。しかし、その種々の形のそれぞれにおいて、それは、その上に置かれるための片状ゲルに用いられるゲル形成溶液に核酸しない物質であろう。上部スペーサー16は、ガラス板間に挿入され図示された位置に置かれる。片状ゲルの溶液は次に加えられて、保護層25の上に層を形成し、保護層は片状ゲル溶液とスラブゲル23との間の全ての接触を実質的に妨げる。

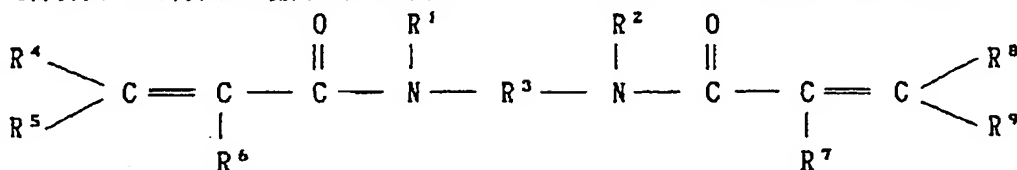
上部スペーサー16は、中心のクロスバー29により接続され、スペーサーの各端から下方にぶらさがった2個の脚部27、28よりなる。上部スペーサー16は、十分に遠く

まで挿入されて、保護層25と片状ゲル溶液26との間の界面30に脚部27、28が延在し、各側面で片状ゲル溶液の層に不連続を残す。図示された態様において、小さなクリアランス31、32は、上部スペーサーの脚部27、28と、肩部21、22の上の側面スペーサー14、15の部分との間に残されることが分かる。これらのクリアランスは、側面スペーサー14、15を乱すことなく上部スペーサー24の挿入及び引抜きの容易さを増すのみの機能を果たす。一度上部スペーサー16が所定の位置におかれたならば、これらのクリアランス領域の片状ゲル溶液は、吸引して除かれるか又は単に放置される。

第2図に示されるように上部スペーサーが所定の位置に置かれると、片状ゲル溶液は放置してゲルに固化される。ゲルが十分に形成されると、上部スペーサー16は除かれる。次に、保護層25は、それ自体除かれるか又はそれが電氣的に絶縁されるならばその場に残される。何れの場合でも目的は、2種のゲルの間に一つのゲルを他のゲルから電氣的に絶縁する介在バリアーを残すことである。もし保護層25が液体の形ならば、それは空けらるか又は吸引されて、2種のゲルの間に空間を残す。例えば、水性ゲルでは、保護層はゲル又はゲル形成溶液の何れかと実質的に不混和性の非極性液体であろう。水より重いクロロホルム及び他の非極性有機溶媒又は油が、この点で使用されよう。又、保護層は、低融点の固体好ましくは約25℃から約75℃の範囲内の融点を有するものであろう。このような材料は、液体の形でスラブゲルの上部の縁24を超えて注がれ、次に冷却され、片状ゲル溶液が加えられる前に固化せしめられる。一度後者が固化すると、システムは再加熱されて保護層をその融点より高くもう一度加熱し、それを液状の形の戻してそれは注がれるか又は吸引できる。この範囲内の沸点を有するステアリン酸メチル及び他の物質がこのような方法に適している。他の方法は、水以外の溶媒に可溶の固体又は重合体の使用である。材料の除去は、従って適切な溶媒を加え得られた溶液を洗い流すことにより行なわれる。さらに他の方法は、可撓性の固体材料例えばゴムの片の使用があり、それは片状ゲル溶液の注入前にスラブゲルの頂部の上に置き、次に片状ゲル溶液が固化し上部スペーサー16除かれたならば引き出される。ゴム又は他の固体材料は、もちろんスラブゲルと片状ゲル溶液との間の接触を防ぐために両方のガラス板に対して横方向にシールできるほど十分に広くなければならない。

前述のように、もし保護層25が片状ゲル溶液とスラブゲルとの間の混合を防ぐその能力に加えて、電氣的に絶縁する材料から作られるならば、それは所定の場所に残すことができる。その結果は、空気よりむしろ固体又は液体の絶縁層であろう。何れの場合でも、結果は2種のゲル間の電流の透過に対するバリアーであり、一端から他端への片状ゲル間に課された全ての電位が片状ゲルそれ自体にその十分な効果を働かせることであろう。上部

第4b図では、片状ゲル26はその初めの位置に放置され、新しい介在ゲル48がそれとスラブゲル23との間に形成される。この介在するゲル48は、導電性であり、しかもそれを通る溶質の泳動を行なわせてスラブゲル23に入らせることができる材料から形成される。この介在ゲルは、適切なゲル形成溶液が片状ゲルの下面に達するか又は超えるまで片状ゲル26の何れかの側面でゲルの囲みに



本発明に従って用いられるゲルは、二次元電気泳動分離に好適に用いられるゲルの任意の組合せである。ゲルは、一般にポリアクリルアミドゲル、澱粉ゲル、アガールゲルなどを含む水性ゲルであり、種々のゲル濃度及び多孔度、均一な濃度又は勾配、一定の又は勾配のpHを用い、可溶化剤を用い又は用いることなく或いは用いるときには種々のタイプ及び濃度の可溶化剤を用い、そして連続又は不連続のバッファースystemを使用する。これらのシステムが用いられる種々の適用の中で、第一の次元が等電点電気泳動であり、第二の次元が一定pH電気泳動である分離がある。システムは、又第一の次元が尿素の存在下でなされる分離に用いられる。この点において、下記の式の橋かけ結合剤により橋かけ結合したポリアクリルアミドゲルが特に有用である。

特に有用なシステムでは、片状ゲルは、ジアクリリルピペラジンにより橋かけ結合したポリアクリルアミドゲルであり、蛋白可溶化量で尿素を含み、そしてジアクリリルピペラジンにより橋かけ結合したポリアクリルアミドゲルであるスラブゲルは、尿素の代わりに又はそれに加えて、蛋白可溶化量でナトリウムドデシルサルフェートを含む。

何れの場合において用語「蛋白可溶化量」は、サンプル中の蛋白を水性相に可溶にする任意の量を示すのにここでは用いられる。尿素の場合では、量は一般に約2M～約25M好ましくは約8M～約10Mに及ぶ。ナトリウムドデシルサルフェートの場合では、量は一般に約0.03重量%～約3重量%の及ぶ。他の可溶化剤は、ホルムアミド及び酢酸を含む。適切な可溶化剤及びその最適な量の選択は、分離される蛋白又は溶質の性質及びサンプル中のそれらの濃度に応じて変化し、そして当業者の技術の範囲内に十分に入る。

前述は、説明のために主として述べられた。ここに記載された材料、方法及び操作条件の変化、修正及び置換は、本発明の趣旨及び範囲から離れることなくできることは当業者にとり容易に明らかであろう。

【発明の効果】

本発明の二次元電気泳動用プレキャストゲルシステムによれば、ゲル及び溶質ゾーンの歪みや乱れが生じることがなく、複雑な蛋白混合物等を容易且つ正確に分離できる。

【図面の簡単な説明】

第1図は、本発明によるゲルを注型し保持するのに用いられるのに適したゲルの囲いの分解透視図である。

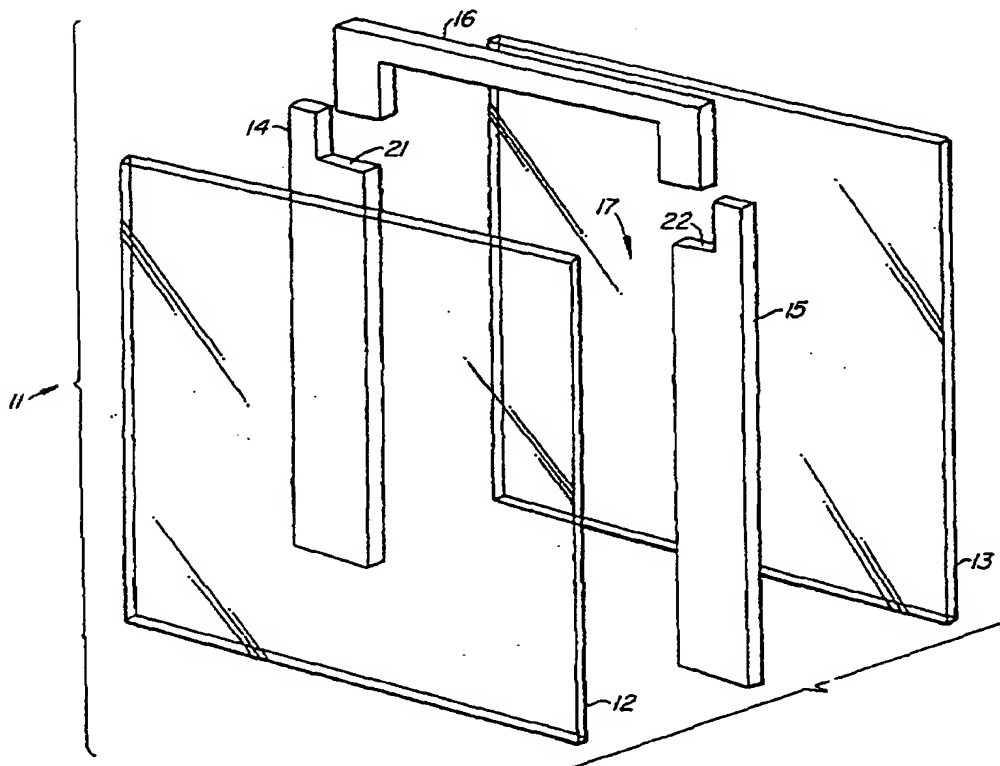
第2図は、第二の次元のゲルの注型直後の一枚の支持板を除いた、第1図のゲルの囲いで製造された二次元ゲルシステムの正面図である。

第3図は、分離の第一の段階注の再び板の一枚を除いた第2図のゲルシステムを示す。

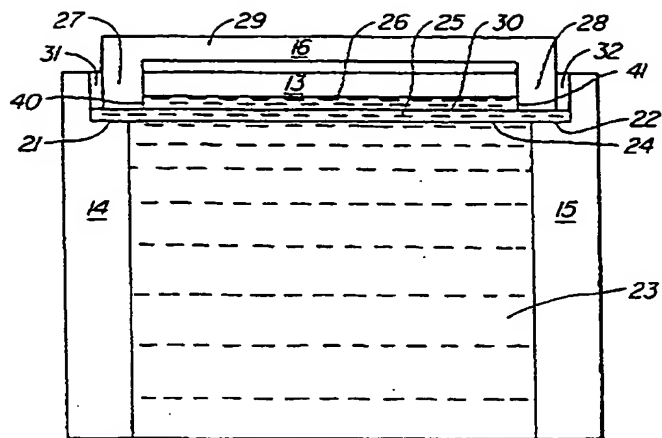
第4a及び4b図は、第二の段階の開始の条件の第2及び3図のゲルシステムを示す。

- 11……ゲルの囲み、12……ガラス板
- 13……ガラス板、14……側面スペーサー
- 15……側面スペーサー、16……上部スペーサー
- 17……内部空間、21……肩部
- 22……肩部、23……スラブゲル
- 24……23の上部の縁、25……保護層
- 26……片状ゲル溶液、27……16の脚部
- 28……16の脚部、29……クロスバー
- 30……界面、31……クリアランス
- 32……クリアランス、40……26の端
- 41……26の端、42……介在空間
- 43……片、44……片
- 45……電解質貯槽、46……電解質貯槽
- 47……ゾーン、48……介在ゲル
- 50……26の上部縁、51……23の下部縁

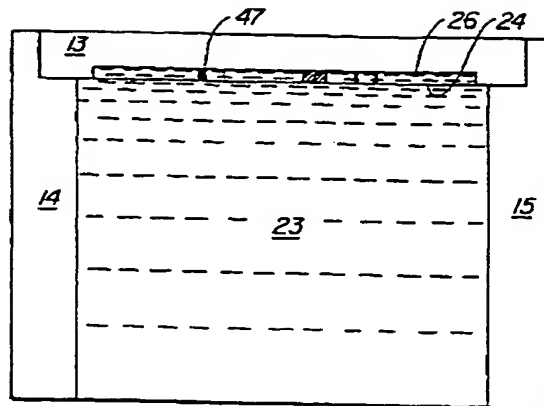
【第1図】



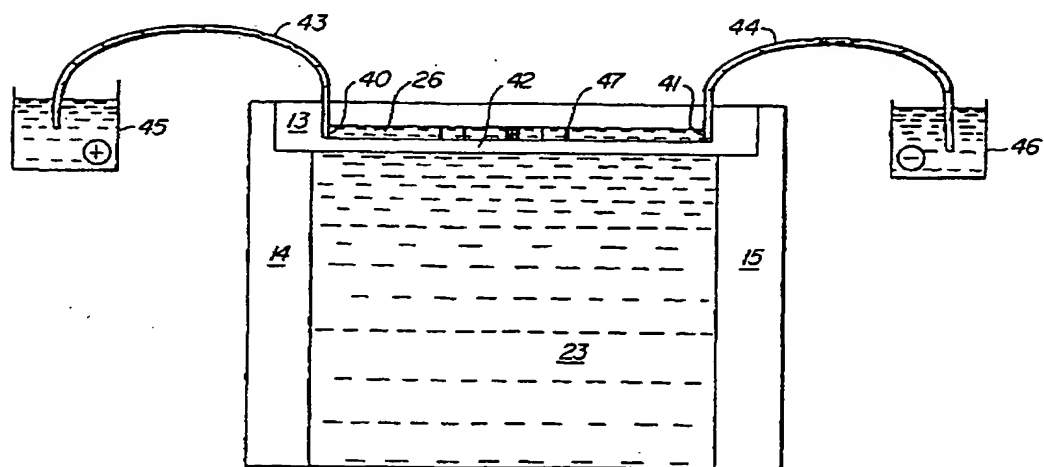
【第2図】



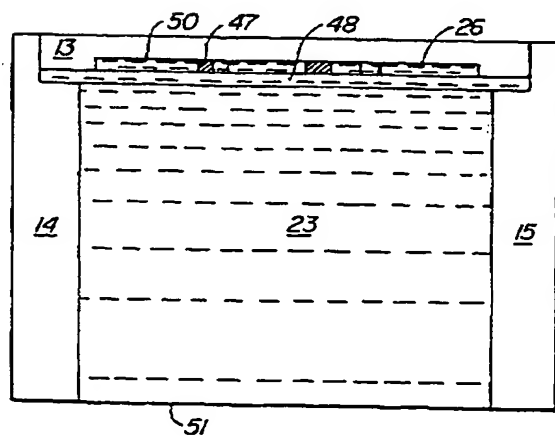
【第4 a 図】



【第3図】



【第4 b 図】



JAPANESE [JP,2701943,B]

CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD EFFECT OF THE INVENTION TECHNICAL
PROBLEM MEANS DESCRIPTION OF DRAWINGS DRAWINGS

[Translation done.]

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CLAIMS

(57) [Claim(s)]

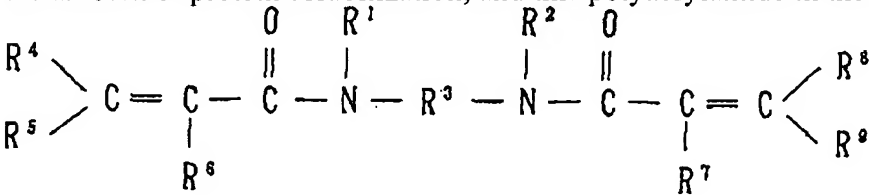
[Claim 1] It is the pre cast two-dimensional-electrophoresis gel system which is mutually separated on it with the non-conductive ingredient which it consists of the first gel of the form of a long and slender piece, and the second gel of the form of slab, and the aforementioned first and the second gel are held on a single gel support means, is immobilization at a room temperature further, and moreover has the melting point between about 25 degrees C and about 75 degrees C.

[Claim 2] It is the pre cast two-dimensional-electrophoresis gel system which it consists of the first gel of the form of a long and slender piece, and the second gel of the form of slab, and the aforementioned first and the second gel are held on a single gel support means, is immobilization at a room temperature further, and is moreover mutually separated at the room temperature on it in the solvent of nonaqueous nature with the meltable non-conductive ingredient.

[Claim 3] The pre cast two-dimensional-electrophoresis gel system according to claim 1 or 2 by which this gel support means consists of an parallel support plate substantially [a lot].

[Claim 4] The pre cast two-dimensional-electrophoresis gel system according to claim 1 or 2 which this gel support means consists of an parallel support plate substantially [a lot], and leaves the open path which makes the layer in which the aforementioned removal is possible, and which is insulated electrically remove from this support plate, without arranging the aforementioned first and the second gel between these support plates, and disturbing the aforementioned first and the second gel.

[Claim 5] The aforementioned first and the second gel are polyacrylamide gels, the second aforementioned gel contains [the first aforementioned gel] the sodium dodecyl sulfate of the amount of protein solubilization including the urea of the amount of protein solubilization, and this polyacrylamide in the first aforementioned gel is a formula.



(R1, R2, and R3 are specified as follows among a formula, namely, R1 and R2 are C1-C5 alkyls independently. R3) Are C1-C8 alkylene, or combine;, or R1 and R2, and formation Perilla frutescens (L.) Britton var. crispa (Thunb.) Decne. of the C1-C8 alkylene is carried out. R3 is C1-C8 alkylene, or combine; or R1 with R3, form the saturation hydrocarbyl radical of 3-10 carbon atoms, and it forms N content ring together with N atom which R1 and R3 have combined. And R2 is C1-C5 alkyl, or;, or R1 and R2 are combined with R3. The saturation hydrocarbyl radical of 7-15 carbon atoms is formed, it forms two N content rings together with N atom, and;, and R4, R5, R6, R7, R8 and R9 are chosen from the group which consists independently of H and C1-C5 alkyl.

The pre cast two-dimensional-electrophoresis gel system according to claim 1 or 2 which is carrying out cross linkage to the compound which ****, and the cross linkage agent.

[Claim 6] In the approach of carrying out casting of the two-dimensional-electrophoresis gel, it leaves the tooth space as for which this approach was vacant between these support plates along the edge which formed the first gel between the support plates of the (a) lot, and the first aforementioned gel exposed.;

(b) Form the first layer which adjoins the edge which put the non-conductive ingredient on the tooth space as for which the above was vacant, and the above of this slab exposed. This non-conductive ingredient is a solid-state at a room temperature, it puts on the tooth space as for which moreover has the melting point between about 25 degrees C and about 75 degrees C, and the above [: (c) gel formation liquid] was vacant, the second long and slender layer which adjoins the first aforementioned layer is formed, and this matter and this gel formation liquid are immiscibility

substantially.;

(d) How to form the second aforementioned layer in long and slender gel, and heat and conductive (e) this ingredient to this melting point or temperature higher than it, and become by picking out this center of the condition of a liquid from this support plate.

[Claim 7] Arrangement of the two-dimensional-electrophoresis gel which consists of the first gel of the form of the long and slender piece which has a long and slender configuration, and the second gel of the form of slab is offered. the approach two dimensional electrophoresis separates a sample into a component -- setting -- this approach -- (a) -- It is held on a gel support means with single aforementioned first and second gel, and the mediation field of the ingredient which the aforementioned first and the second gel are immobilization at a room temperature, and moreover has the melting point between about 25 degrees C and about 75 degrees C dissociates.;

(b) Put this sample on the end on the first aforementioned gel.;

(c) Apply electric field in the direction parallel to the aforementioned long and slender configuration between the first aforementioned gel, perform electrophoresis separation of this component of this sample there, and consider as a zone.;

(d) Heat this non-conductive ingredient more highly than this melting point.;

(e) While being in the condition of a liquid, it is drawing about this non-conductive ingredient.;

(f) How to consist of performing electrophoresis separation of this zone of the second aforementioned gel, applying electric field in the direction which the first aforementioned gel is moved toward the second aforementioned gel, and the aforementioned first and the second gel are contacted directly, and crosses; and the long and slender configuration of (g) above between both the aforementioned first and the second gel.

[Claim 8] Arrangement of the two-dimensional-electrophoresis gel which consists of the first gel of the form of the long and slender piece which has a long and slender configuration, and the second gel of the form of slab is offered. the approach two dimensional electrophoresis separates a sample into a component -- setting -- this approach -- (a) -- It is held on a gel support means with single aforementioned first and second gel, and the mediation field of the ingredient which the aforementioned first and the second gel are solid-states at a room temperature, and moreover has the melting point between about 25 degrees C and about 75 degrees C dissociates.;

(b) Put this sample on the end on the first aforementioned gel.;

(c) Apply electric field in the direction parallel to the aforementioned long and slender configuration between the first aforementioned gel, perform electrophoresis separation of this component of this sample there, and consider as a zone.;

(d) Heat this non-conductive ingredient more highly than this melting point.;

(e) While there is a condition of a liquid, a conductive gel formation liquid permutes this non-conductive ingredient.;

(f) How to consist of performing electrophoresis separation of this zone of the second aforementioned gel, applying electric field in the direction which forms this gel formation liquid in conductive gel, and crosses; and the long and slender configuration of (g) above between both the aforementioned first and the second gel.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[Industrial Application]

Especially this invention relates to the process and usage of gel which are used for 2-dimensional gel separation about 2-dimensional gel electrophoresis.

[Description of the Prior Art]

Two dimensional electrophoresis is widely used for separating complicated protein mixture. Two dimensional electrophoresis brings about decomposition capacity very higher than it which is obtained by separation of a single stage by producing separation based on the property of two sorts of different groups continuously.

A separation parameter is combined in various ways using a technique. For example, separation based on a charge is performed in the first phase, and then separation based on molecular weight is performed in the second phase. Similarly, the separation in other concentration of the separation in one gel concentration, next the same gel is made. gradual change of pH according to two-step use as other examples, and homogeneity gel -- isoelectric focusing accompanied by the inclination electrophoresis of **** porosity which is next a buffer system [****] in the discontinuous buffer system and other phases in use of two sorts of concentration of porous inclination gel, two sorts of different protein resolvers, or the same solvent, and one phase, and homogeneity. Such a technique makes separation of the mixture of a blood serum or the protein of a cell, bacterial protein, non-histone chromatin protein, ribosome protein, ribonucleoprotein, and liposome protein, a nucleic acid, and the same matter perform, and is closed.

Or the fundamental approach of a 2-dimensional system is long and slender, it starts in the single dimension separation by what has rod-like gel, for example, the diameter of 5.0mm order, and in accordance with the shaft of gel, migration and separation produce it until a solute or protein is distributed in the zone in which it is located along with the die length of a rod. Next, a rod is arranged along one edge of slab gel, and migration of the second dimension of a solute is performed to slab gel from each zone in the direction which crosses the shaft of the gel of a rod.

The difficulty which encounters in this type of way is related with migration of the gel of the shape of a rod after separation of the first dimension arises, in order to separate the second dimension. Generally the gel of a rod produces separation of the first dimension within tubing by which casting was carried out at first. If separation of the first dimension is completed, the rod of the gel which has a solute zone will be picked out from tubing, physical means, for example, extract, and will be placed along the edge which slab gel exposed next. Even if these actuation needs delicate handling and it uses cautions very much, gel was spoiled and risk of producing the distortion and turbulence of a solute zone remains. Once it is extracted, the gel of a rod will be arranged as appropriately as slab gel, and will fully be contacted for the purpose of both migration of a solute of not being blocked between an electric continuity and gel.

These are other sources of an error and spoil repeatability. The time amount which handling and arrangement of the gel of a rod take at the difficulty list of processing in addition to the possibility of loss of inaccuracy and repeatability will reduce an operator's efficiency, and will cause the time amount and the loss of data to which recovery does not attach any fatal errors. Although some people use the piece of gel for separation of the first dimension, they encounter loss of the same difficulty, an error, and repeatability.

[The means for solving a technical problem]

It is together put on the single gel base material with which, as for this invention, the phase of both separation produces the gel of the first and the second dimension about a 2-dimensional pre cast (precast) gel system. It is the enclosure which consists of a plate of a single group, and a base material has a suitable spacer, between plates, it prepares space and specifies the thickness of gel. Casting of both the gels of the first and the second dimension is carried out, and they are held between plates. The gel of the second dimension is slab which occupies a part of space between plates, and on the other hand, the first dimension is a piece located in the remaining space in parallel at slab, and it has the layer in

which the removal which divides gel is possible and which is insulated electrically. Moreover, a base material is the gel backing which consists of a sheet of elastics, gel bond, or other charges of supporting material, and casting of both the gels of the first and the second dimension is carried out on it, and it has among them the insulating layer which is the layer of an insulating material, or the tooth space of air.

While separation of the first dimension follows, puts a sample on the end of piece-like gel and insulates two sorts of gels electrically mutually. Electric field are applied in the die-length direction between piece-like gels, electric contact is carried out [the layer insulated electrically next] for drawing and two sorts of gels along with those perfect die length, and it is carried out by applying electric field in the direction which crosses the first it next among both. the electric contact between two sorts of gels [in / a solid-state, a liquid, or a gas is sufficient as the layer insulated electrically, and / separation of the second dimension] -- direct contact -- or it is attained by insertion of a conductive layer by any of a twist they are. This invention abolishes the need of moving a rod or piece-like gel to other things from one support enclosure among two sorts of phases of separation, and reduces loss of gel, and the risk [zone] of distortion by that cause, and makes generating of inaccuracy and a constant error decrease.

Probably, other purposes, advantages, and features of this invention will be clear from the following description.

Fig. 1 is the decomposition perspective drawing of the enclosure of gel suitable for being used for carrying out casting of the gel by this invention, and holding it.

Fig. 2 is a front view of the 2-dimensional gel system manufactured in the enclosure of the gel of Fig. 1 except the support plate of one sheet just behind the casting of the gel of the second dimension.

Fig. 3 shows the gel system of Fig. 2 in the first [of separation] phase excluding one plate again.

the -- 4b Fig. shows the gel system of the 2nd and 3 Fig. of the conditions of initiation of the second stairway to 4a **.

According to this invention, the long and slender gel of the form of a piece is used for separation of the first dimension instead of the gel of the shape of a rod of a Prior art, or the separated piece of gel. Casting of the piece-like gel is carried out between support plates in the same way as slab gel as a shallow layer on slab gel. This is made after slab gel is formed preferably. becoming instead of [of a barrier layer], if a solid-state, a liquid, or a gas is sufficient as the insulating layer between two, and it exists in the casting of piece gel or (it works in this case as a barrier which prevents contact of the fluid between two sorts of gels again) casting of the piece-like gel is carried out -- it is in any.

Various layers and the enclosure of gel are fabricated in a way in which an insulating layer is removed when separation of the first dimension is completed once, and consider as such magnitude in it. Generally this is made by preparing the opened path which connects the exterior of the enclosure of gel, and the field of an insulating layer. or [being pushed on the method of outside by a path's being the form of the surrounding path clearance of the end of piece-like gel, or both ends preferably, and eliminating an insulating layer there] -- or being removed -- it is in any.

An attached drawing offers detailed drawing of one explanatory mode of this invention.

Fig. 1 shows the enclosure 11 of the gel of the decomposed form suitable for being used for the casting of the gel for the stairway of both electrophoresis, and use. The component part of an enclosure contains the glass plate of two sheets, the side-face spacers 14 and 15 of a lot, and the up spacer 16. The side-face spacers 14 and 15 determine the thickness [as / in the enclosure of the conventional slab gel] of the gel by which prepares space among glass plates 12 and 13, and casting is carried out by that cause between plates. The illustrated side-face spacer has the thickness of homogeneity, and brings about the gel of the thickness of homogeneity similarly. Moreover, the side-face spacer, for example, a wedge-shaped spacer, which has the thickness which changes along with the die length of a spacer may be perpendicularly used in order to change the thickness of gel.

The side-face spacers 14 and 15 and the glass plates 12 and 13 of each other are held by arrangement of the type of sandwiches by the clamp (not shown) along each side face. The space 17 between a glass plate and a side-face spacer is filled with a gel solution, it is made to solidify according to the conventional approach by gel, and an enclosure is mounted at right angles to the stand for casting (not shown). Other instruments required of forming a suitable clamp, the stand for casting, and gel are used widely conventionally, and are available.

The side-face spacers 14 and 15 are fixed to a predetermined location by the clamp. However, the up spacer 16 can be removed and is used into the casting of piece-like gel. The use is related with the 2nd drawing 2 Fig., and is shown still more clearly.

In Fig. 2, the glass plate 12 ahead of Fig. 1 is removed for plainness. The side-face spacers 14 and 15 of 13 or 2 back glass plates and the removable up spacer 16 remain in drawing.

It should return to Fig. 1 and the side-face spacers 14 and 15 should be noticed about equipping the internal space 17 with the other shoulders 21 and 22, respectively. In this mode, these shoulders attach the mark of the level of the liquid for pouring out the solution with which the slab gel 23 (Fig. 2) is formed from it. The edge 24 of the upper part of slab gel follows, and is located at the same flat surface as these shoulders. Although casting of the slab gel is carried out, the

enclosure 11 of gel is assembled without including the up spacer 16, and a gel solution is poured out and solidified by the level of shoulders 12 and 22.

Once the slab gel 23 is fully formed, a protective layer 25 will be placed on it and will carry out the seal of the edge 24 of the upper part. This protective layer can change a form and a presentation so that it may be explained below. However, in each of the various forms, it is matter which does not carry out a nucleic acid to the gel formation solution used for the piece-like gel for being placed on it. The up spacer 16 is put on the location which was inserted between glass plates and illustrated. The solution of piece-like gel is added to a degree, a layer is formed on a protective layer 25, and a protective layer bars substantially all contact between a piece-like gel solution and the slab gel 23.

The central crossbar 29 connects and the up spacer 16 consists of the two legs 27 and 28 which hung down caudad from each edge of a spacer. It is fully inserted to a long distance, the legs 27 and 28 extend in the interface 30 between a protective layer 25 and the piece-like gel solution 26, and the up spacer 16 leaves discontinuity to the layer of a piece-like gel solution on each side face. In the illustrated mode, it turns out that the small path clearance 31 and 32 is left behind between the legs 27 and 28 of an up spacer, and the part of the side-face spacers 14 and 15 on shoulders 21 and 22. Such path clearance achieves the function of only increasing insertion of the up spacer 24, and the ease of drawing, without disturbing the side-face spacers 14 and 15. or [that attract the piece-like gel solution of these path clearance fields, and the up spacer 16 is once removed also in a position] -- or it is only left.

If an up spacer is put on a position as shown in Fig. 2, a piece-like gel solution will be left and will be solidified by gel. If gel is fully formed, the up spacer 16 will be removed. Next, a protective layer 25 will be left behind on that occasion, if it removes in itself or it is insulated electrically. In any case, the purpose is leaving the mediation barrier which insulates one gel from other gels electrically among two sorts of gels. supposing a protective layer 25 is the form of a liquid -- it -- vacating -- **** -- or it is drawn in and leaves space among two sorts of gels. For example, in aquosity gel, a protective layer is an immiscible nonpolar liquid substantially with any of gel or a gel formation solution they are. Chloroform and other nonpolar organic solvents, or an oil heavier than water will be used at this point. moreover, a protective layer -- the solid-state of a low-melt point point -- probably, it has the melting point within the limits of about 25 to about 75 degrees C preferably. It is poured across the edge 24 of the upper part of slab gel in the form of a liquid, and then is cooled, and before a piece-like gel solution is added, it is made to solidify such an ingredient. or [that a system will be reheated, will heat a protective layer once again more highly than the melting point, a liquefied form will return it, and it will be poured out once the latter solidifies] -- or it can draw in. The methyl stearate and other matter which have the boiling point within the limits of this fit such an approach. Other approaches are use of a solid-state meltable to solvents other than water, or a polymer. Removal of an ingredient is performed by flushing the solution which follows and was able to add the suitable solvent. The approach of further others has use of a flexible solid material, for example, the piece of rubber, and it places on the crowning of slab gel before impregnation of a piece-like gel solution, and it will be pulled out, if a piece-like gel solution next solidifies and it removes up spacer 16. Rubber or other solid materials must fully be so large that they can carry out a seal to a longitudinal direction to both glass plates in order to, prevent contact between slab gel and a piece-like gel solution, of course.

As mentioned above, if made from the ingredient insulated electrically in addition to the capacity for a protective layer 25 to prevent mixing between a piece-like gel solution and slab gel, it can leave it to a predetermined location. The result is the insulating layer of a solid-state or a liquid more nearly rather than air. A result is a barrier to transparency of the current between two sorts of gels, and, in any case, it will be that all the potentials imposed between the piece-like gels from an end to the other end use the sufficient effectiveness to piece-like gel itself. picking of the up spacer 16 -- removing -- two edges 40 and 41 of the piece-like gel 26 are exposed, this helps those connections with an electrode buffer, and electrophoresis separation is produced.

Fig. 3 is drawing of one method of performing electrophoresis of a first stage story. In this mode, except for a protective layer 25, the edge where gel was exposed is dried with air, and the intervening space 42 is occupied with air. The pieces 43 and 44 of the adsorption ingredient made to become wet with an electrolytic solution are contacted with each two edge 40 and 41 of the piece-like gel 26. The edge at which each piece is opposed to each other is imposed by the means of the former [potential], while being immersed at the electrolyte tanks 45 and 46.

The sample which should be separated is carried on the piece-like gel 26 by the conventional means of arbitration. For example, before a sample is placed so that the pieces 43 and 44 of adsorption of two sheets may contact the piece-like gel 26, it is placed at these edges of one sheet. The electrophoresis of a first stage story is produced next, and a solute migrates in the direction of the length of piece-like gel, and forms the zone separated along with the piece-like gel 26 as shown.

Once separation of desired extent arises at the first dimension, two sorts of gels will carry out electric contact, and the solute of all single zones will be separated mutually. the [the 4a and] -- 4b Fig. shows the various methods of attaining

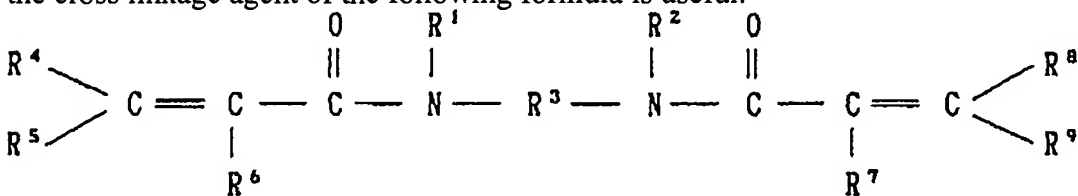
this. the -- in 4a Fig., the piece-like gel 26 is physically depressed until it fully contacts the slab gel 23 by the edge 24 of the upper part of the slab gel which now forms the interface between two sorts of gels. It must take care that there is no prehension of air bubbles among two sorts of gels.

the -- in 4b Fig., the piece-like gel 26 is left in the first location, and the new mediation gel 48 is formed between it and the slab gel 23. This intervening gel 48 is conductivity and is formed from the ingredient which can be made to be able to perform migration of the solute which moreover passes along it, and can be made to go into the slab gel 23. This mediation gel fully contacts among two sorts of gels, without catching air bubbles, if it can form whether a suitable gel formation solution arrives at the inferior surface of tongue of piece-like gel, and by flowing into the enclosure of gel on which side face of the piece-like gel 26 until it exceeds and solidifies. The mediation gel 48 is the thing of other ingredients of the arbitration which brings about the same ingredients as slab gel, or these results.

The above-mentioned approach is equally [to the gel by which the polymerization was carried out on plastics backing or gel bond] applicable.

Once electric contact is made between the piece-like gel 26 and the slab gel 23, in order to perform electrophoresis of a second stage story, the edge 50 of the upper part of piece-like gel and the edge 51 of the lower part of slab gel will contact an electrode buffer, will be placed, and will bring about the migration of the lower part from piece-like gel to slab gel. This is marketed widely and use of the well-known conventional slab electrophoresis cel can attain it to this contractor. Such one example of a cel is indicated by U.S. Pat. No. 4575040, and is quoted as bibliography here. Once separation of desired extent arises at the second dimension, slab gel will be taken out from an enclosure and will be analyzed according to the conventional approach. Generally these contain any of the quantitative or qualitative measurement of a solute pattern which were obtained by dyeing of gel and desiccation, and the degree they are.

The gel used according to this invention is the combination of the arbitration of the gel used suitable for two-dimensional-electrophoresis separation. Gel is aquosity gel which generally contains polyacrylamide gel, starch gel, agger gel, etc., and when using using pH of various gel concentration and porosity, uniform concentration or inclination, and regularity or inclination, without using, using a solubilizing agent, the buffer system of continuation or discontinuity is used for it, using various types and the solubilizing agent of concentration. There is separation whose first dimension is isoelectric focusing and whose second dimension is fixed pH electrophoresis in the various application for which these systems are used. A system is used for the separation by which the dimension of the first is made under existence of a urea again. In this point, especially the polyacrylamide gel that carried out cross linkage by the cross linkage agent of the following formula is useful.



(R1, R2, and R3 are specified as follows among a formula.) Namely, R1 and R2 are C1-C5 alkyls independently, R3 is C1-C8 alkylene, or, or R1 and R2 are combined. Form C1-C8 alkylene, and R3 is C1-C8 alkylene, or combine; or R1 with R3, form the saturation hydrocarbyl radical of 3-10 carbon atoms, and it forms N content ring together with N atom which R1 and R3 have combined. R2 is C1-C5 alkyl, or, or R1 and R2 are combined with R3, the saturation hydrocarbyl radical of 7-15 carbon atoms is formed, and it forms two N content rings together with N atom. And, and R4, R5, R6, R7, R8 and R9 It is chosen out of the group which consists independently of H and C1-C5 alkyl.

In an especially useful system, piece-like gel is the polyacrylamide gel which carried out cross linkage by the JIAKURIRIRU piperazine, and instead a urea boils the slab gel which is the polyacrylamide gel which carried out cross linkage by the JIAKURIRIRU piperazine, including a urea in the amount of protein solubilization, or, in addition to it, sodium dodecyl sulfate is included in the amount of protein solubilization.

In the case of which, the vocabulary "the amount of protein solubilization" is used here that the amount of the arbitration which makes the protein in a sample meltable to an aquosity phase is shown. the case of a urea -- an amount -- general -- about 2M- about 25 -- M -- desirable -- about 8M- about 10 -- M is attained to. the case of sodium dodecyl sulfate -- an amount -- general -- about 0.03 % of the weight - about 3 % of the weight -- it reaches. Other solubilizing agents contain a formamide and an acetic acid. Selection of a suitable solubilizing agent and its optimal amount changes according to the properties of the protein separated or a solute, and those concentration in a sample, and fully enters within the limits of this contractor's technique.

The above-mentioned was mainly described for explanation. Probably, it will be easily [for this contractor] clear that a

permutation's [change of the ingredient indicated here, an approach, and an operating condition, correction, and] it can do, without separating from the m...ng and the range of this invention.

[Effect of the Invention]

According to the pre cast gel system for two dimensional electrophoresis of this invention, the distortion or turbulence of gel and a solute zone do not arise, and complicated protein mixture etc. can be separated easily and correctly.

[Translation done.]

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TECHNICAL FIELD

[Industrial Application]

Especially this invention relates to the process and usage of gel which are used for 2-dimensional gel separation about 2-dimensional gel electrophoresis.

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EFFECT OF THE INVENTION

[Effect of the Invention]

According to the pre cast gel system for two dimensional electrophoresis of this invention, the distortion or turbulence of gel and a solute zone do not arise, and complicated protein mixture etc. can be separated easily and correctly.

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TECHNICAL PROBLEM

[Description of the Prior Art]

Two dimensional electrophoresis is widely used for separating complicated protein mixture. Two dimensional electrophoresis brings about decomposition capacity very higher than it which is obtained by separation of a single stage by producing separation based on the property of two sorts of different groups continuously.

A separation parameter is combined in various ways using a technique. For example, separation based on a charge is performed in the first phase, and then separation based on molecular weight is performed in the second phase. Similarly, the separation in other concentration of the separation in one gel concentration, next the same gel is made. gradual change of pH according to two-step use as other examples, and homogeneity gel -- isoelectric focusing accompanied by the inclination electrophoresis of **** porosity which is next a buffer system [****] in the discontinuous buffer system and other phases in use of two sorts of concentration of porous inclination gel, two sorts of different protein resolvers, or the same solvent, and one phase, and homogeneity. Such a technique makes separation of the mixture of a blood serum or the protein of a cell, bacterial protein, non-histone chromatin protein, ribosome protein, ribonucleoprotein, and liposome protein, a nucleic acid, and the same matter perform, and is closed.

Or the fundamental approach of a 2-dimensional system is long and slender, it starts in the single dimension separation by what has rod-like gel, for example, the diameter of 5.0mm order, and in accordance with the shaft of gel, migration and separation produce it until a solute or protein is distributed in the zone in which it is located along with the die length of a rod. Next, a rod is arranged along one edge of slab gel, and migration of the second dimension of a solute is performed to slab gel from each zone in the direction which crosses the shaft of the gel of a rod.

The difficulty which encounters in this type of way is related with migration of the gel of the shape of a rod after separation of the first dimension arises, in order to separate the second dimension. Generally the gel of a rod produces separation of the first dimension within tubing by which casting was carried out at first. If separation of the first dimension is completed, the rod of the gel which has a solute zone will be picked out from tubing, physical means, for example, extract, and will be placed along the edge which slab gel exposed next. Even if these actuation needs delicate handling and it uses cautions very much, gel was spoiled and risk of producing the distortion and turbulence of a solute zone remains. Once it is extracted, the gel of a rod will be arranged as appropriately as slab gel, and will fully be contacted for the purpose of both migration of a solute of not being blocked between an electric continuity and gel.

These are other sources of an error and spoil repeatability. The time amount which handling and arrangement of the gel of a rod take at the difficulty list of processing in addition to the possibility of loss of inaccuracy and repeatability will reduce an operator's efficiency, and will cause the time amount and the loss of data to which recovery does not attach any fatal errors. Although some people use the piece of gel for separation of the first dimension, they encounter loss of the same difficulty, an error, and repeatability.

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MEANS

[The means for solving a technical problem]

It is together put on the single gel base material with which, as for this invention, the phase of both separation produces the gel of the first and the second dimension about a 2-dimensional pre cast (precast) gel system. It is the enclosure which consists of a plate of a single group, and a base material has a suitable spacer, between plates, it prepares space and specifies the thickness of gel. Casting of both the gels of the first and the second dimension is carried out, and they are held between plates. The gel of the second dimension is slab which occupies a part of space between plates, and on the other hand, the first dimension is a piece located in the remaining space in parallel at slab, and it has the layer in which the removal which divides gel is possible and which is insulated electrically. Moreover, a base material is the gel backing which consists of a sheet of plastics, gel bond, or other charges of supporting material, and casting of both the gels of the first and the second dimension is carried out on it, and it has among them the insulating layer which is the layer of an insulating material, or the tooth space of air.

While separation of the first dimension follows, puts a sample on the end of piece-like gel and insulates two sorts of gels electrically mutually. Electric field are applied in the die-length direction between piece-like gels, electric contact is carried out [the layer insulated electrically next] for drawing and two sorts of gels along with those perfect die length, and it is carried out by applying electric field in the direction which crosses the first it next among both. the electric contact between two sorts of gels [in / a solid-state, a liquid, or a gas is sufficient as the layer insulated electrically, and / separation of the second dimension] -- direct contact -- or it is attained by insertion of a conductive layer by any of a twist they are. This invention abolishes the need of moving a rod or piece-like gel to other things from one support enclosure among two sorts of phases of separation, and reduces loss of gel, and the risk [zone] of distortion by that cause, and makes generating of inaccuracy and a constant error decrease.

Probably, other purposes, advantages, and features of this invention will be clear from the following description.

Fig. 1 is the decomposition perspective drawing of the enclosure of gel suitable for being used for carrying out casting of the gel by this invention, and holding it.

Fig. 2 is a front view of the 2-dimensional gel system manufactured in the enclosure of the gel of Fig. 1 except the support plate of one sheet just behind the casting of the gel of the second dimension.

Fig. 3 shows the gel system of Fig. 2 in the first [of separation] phase excluding one plate again.

the -- 4b Fig. shows the gel system of the 2nd and 3 Fig. of the conditions of initiation of the second stairway to 4a **. According to this invention, the long and slender gel of the form of a piece is used for separation of the first dimension instead of the gel of the shape of a rod of a Prior art, or the separated piece of gel. Casting of the piece-like gel is carried out between support plates in the same way as slab gel as a shallow layer on slab gel. This is made after slab gel is formed preferably. becoming instead of [of a barrier layer], if a solid-state, a liquid, or a gas is sufficient as the insulating layer between two, and it exists in the casting of piece gel or (it works in this case as a barrier which prevents contact of the fluid between two sorts of gels again) casting of the piece-like gel is carried out -- it is in any.

Various layers and the enclosure of gel are fabricated in a way in which an insulating layer is removed when separation of the first dimension is completed once, and consider as such magnitude in it. Generally this is made by preparing the opened path which connects the exterior of the enclosure of gel, and the field of an insulating layer. or [being pushed on the method of outside by a path's being the form of the surrounding path clearance of the end of piece-like gel, or both ends preferably, and eliminating an insulating layer there] -- or being removed -- it is in any.

An attached drawing offers detailed drawing of one explanatory mode of this invention.

Fig. 1 shows the enclosure 11 of the gel of the decomposed form suitable for being used for the casting of the gel for the stairway of both electrophoresis, and use. The component part of an enclosure contains the glass plate of two sheets, the side-face spacers 14 and 15 of a lot, and the up spacer 16. The side-face spacers 14 and 15 determine the thickness [as /

in the enclosure of the conventional slab gel] of the gel by which prepares space among glass plates 12 and 13, and casting is carried out by that cause between plates. The illustrated side-face spacer has the thickness of homogeneity, and brings about the gel of the thickness of homogeneity similarly. Moreover, the side-face spacer, for example, a wedge-shaped spacer, which has the thickness which changes along with the die length of a spacer may be perpendicularly used in order to change the thickness of gel.

The side-face spacers 14 and 15 and the glass plates 12 and 13 of each other are held by arrangement of the type of sandwiches by the clamp (not shown) along each side face. The space 17 between a glass plate and a side-face spacer is filled with a gel solution, it is made to solidify according to the conventional approach by gel, and an enclosure is mounted at right angles to the stand for casting (not shown). Other instruments required of forming a suitable clamp, the stand for casting, and gel are used widely conventionally, and are available.

The side-face spacers 14 and 15 are fixed to a predetermined location by the clamp. However, the up spacer 16 can be removed and is used into the casting of piece-like gel. The use is related with the 2nd drawing 2 Fig., and is shown still more clearly.

In Fig. 2, the glass plate 12 ahead of Fig. 1 is removed for plainness. The side-face spacers 14 and 15 of 13 or 2 back glass plates and the removable up spacer 16 remain in drawing.

It should return to Fig. 1 and the side-face spacers 14 and 15 should be noticed about equipping the internal space 17 with the other shoulders 21 and 22, respectively. In this mode, these shoulders attach the mark of the level of the liquid for pouring out the solution with which the slab gel 23 (Fig. 2) is formed from it. The edge 24 of the upper part of slab gel follows, and is located at the same flat surface as these shoulders. Although casting of the slab gel is carried out, the enclosure 11 of gel is assembled without including the up spacer 16, and a gel solution is poured out and solidified by the level of shoulders 12 and 22.

Once the slab gel 23 is fully formed, a protective layer 25 will be placed on it and will carry out the seal of the edge 24 of the upper part. This protective layer can change a form and a presentation so that it may be explained below.

However, in each of the various forms, it is matter which does not carry out a nucleic acid to the gel formation solution used for the piece-like gel for being placed on it. The up spacer 16 is put on the location which was inserted between glass plates and illustrated. The solution of piece-like gel is added to a degree, a layer is formed on a protective layer 25, and a protective layer bars substantially all contact between a piece-like gel solution and the slab gel 23.

The central crossbar 29 connects and the up spacer 16 consists of the two legs 27 and 28 which hung down caudad from each edge of a spacer. It is fully inserted to a long distance, the legs 27 and 28 extend in the interface 30 between a protective layer 25 and the piece-like gel solution 26, and the up spacer 16 leaves discontinuity to the layer of a piece-like gel solution on each side face. In the illustrated mode, it turns out that the small path clearance 31 and 32 is left behind between the legs 27 and 28 of an up spacer, and the part of the side-face spacers 14 and 15 on shoulders 21 and 22. Such path clearance achieves the function of only increasing insertion of the up spacer 24, and the ease of drawing, without disturbing the side-face spacers 14 and 15. or [that attract the piece-like gel solution of these path clearance fields, and the up spacer 16 is once removed also in a position] -- or it is only left.

If an up spacer is put on a position as shown in Fig. 2, a piece-like gel solution will be left and will be solidified by gel. If gel is fully formed, the up spacer 16 will be removed. Next, a protective layer 25 will be left behind on that occasion, if it removes in itself or it is insulated electrically. In any case, the purpose is leaving the mediation barrier which insulates one gel from other gels electrically among two sorts of gels. supposing a protective layer 25 is the form of a liquid -- it -- vacating -- **** -- or it is drawn in and leaves space among two sorts of gels. For example, in aqueous gel, a protective layer is an immiscible nonpolar liquid substantially with any of gel or a gel formation solution they are. Chloroform and other nonpolar organic solvents, or an oil heavier than water will be used at this point. moreover, a protective layer -- the solid-state of a low-melt point point -- probably, it has the melting point within the limits of about 25 to about 75 degrees C preferably. It is poured across the edge 24 of the upper part of slab gel in the form of a liquid, and then is cooled, and before a piece-like gel solution is added, it is made to solidify such an ingredient. or [that a system will be reheated, will heat a protective layer once again more highly than the melting point, a liquefied form will return it, and it will be poured out once the latter solidifies] -- or it can draw in. The methyl stearate and other matter which have the boiling point within the limits of this fit such an approach. Other approaches are use of a solid-state meltable to solvents other than water, or a polymer. Removal of an ingredient is performed by flushing the solution which follows and was able to add the suitable solvent. The approach of further others has use of a flexible solid material, for example, the piece of rubber, and it places on the crowning of slab gel before impregnation of a piece-like gel solution, and it will be pulled out, if a piece-like gel solution next solidifies and it removes up spacer 16. Rubber or other solid materials must fully be so large that they can carry out a seal to a longitudinal direction to both glass plates in order to, prevent contact between slab gel and a piece-like gel solution, of course.

As mentioned above, if made from the ingredient insulated electrically in addition to the capacity for a protective layer 25 to prevent mixing between a piece-like gel solution and slab gel, it can leave to a predetermined location. The result is the insulating layer of a solid state or a liquid more nearly rather than air. A result is a barrier to transparency of the current between two sorts of gels, and, in any case, it will be that all the potentials imposed between the piece-like gels from an end to the other end use the sufficient effectiveness to piece-like gel itself. picking of the up spacer 16 -- removing -- two edges 40 and 41 of the piece-like gel 26 are exposed, this helps those connections with an electrode buffer, and electrophoresis separation is produced.

Fig. 3 is drawing of one method of performing electrophoresis of a first stage story. In this mode, except for a protective layer 25, the edge where gel was exposed is dried with air, and the intervening space 42 is occupied with air. The pieces 43 and 44 of the adsorption ingredient made to become wet with an electrolytic solution are contacted with each two edge 40 and 41 of the piece-like gel 26. The edge at which each piece is opposed to each other is imposed by the means of the former [potential], while being immersed at the electrolyte tanks 45 and 46.

The sample which should be separated is carried on the piece-like gel 26 by the conventional means of arbitration. For example, before a sample is placed so that the pieces 43 and 44 of adsorption of two sheets may contact the piece-like gel 26, it is placed at these edges of one sheet. The electrophoresis of a first stage story is produced next, and a solute migrates in the direction of the length of piece-like gel, and forms the zone separated along with the piece-like gel 26 as shown.

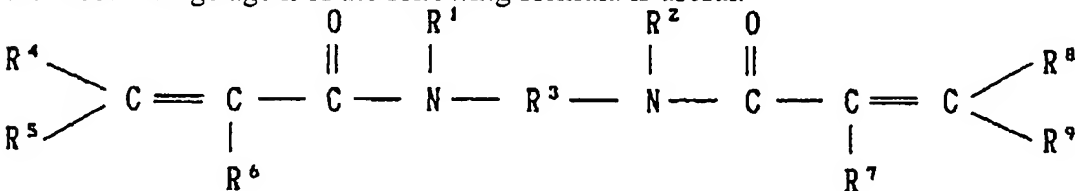
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In the case of which, the vocabulary "the amount of protein solubilization" is used here that the amount of the arbitration which makes the protein in a sample meltable to an aqueous phase is shown. the case of a urea -- an amount -- general -- about 2M- about 25 -- M -- desirable -- about 8M- about 10 -- M is attained to. the case of sodium dodecyl sulfate -- an amount -- general -- about 0.03 % of the weight - about 3 % of the weight -- it reaches. Other solubilizing agents contain a formamide and an acetic acid. Selection of a suitable solubilizing agent and its optimal amount changes according to the properties of the protein separated or a solute, and those concentration in a sample, and fully enters within the limits of this contractor's technique.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

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Fig. 2 is a front view of the 2-dimensional gel system manufactured in the enclosure of the gel of Fig. 1 except the support plate of one sheet just behind the casting of the gel of the second dimension.

Fig. 3 shows the gel system of Fig. 2 of the first phase notes of separation excluding one plate again.

The 4a and 4b Fig. show the gel system of the 2nd and 3 Fig. of the conditions of initiation of the second phase.

11 The enclosure of gel, 12 .. Glass plate

13 A glass plate, 14 .. Side-face spacer

15 A side-face spacer, 16 .. Up spacer

17 A building envelope, 21 .. Shoulder

22 A shoulder, 23 .. Slab gel

The edge of the upper part of 24....23, 25 .. Protective layer

26 A piece-like gel solution, the leg of 27..16

The leg of 28....16, 29 .. Crossbar

30 An interface, 31 .. Path clearance

32 Path clearance, edge of 40..26

The edge of 41....26, 42 .. Mediation space

43 A piece, 44 .. Piece

45 An electrolyte tank, 46 .. Electrolyte tank

47 A zone, 48 .. Mediation gel

The up edge of 50....26, the lower edge of 51..23

[Translation done.]

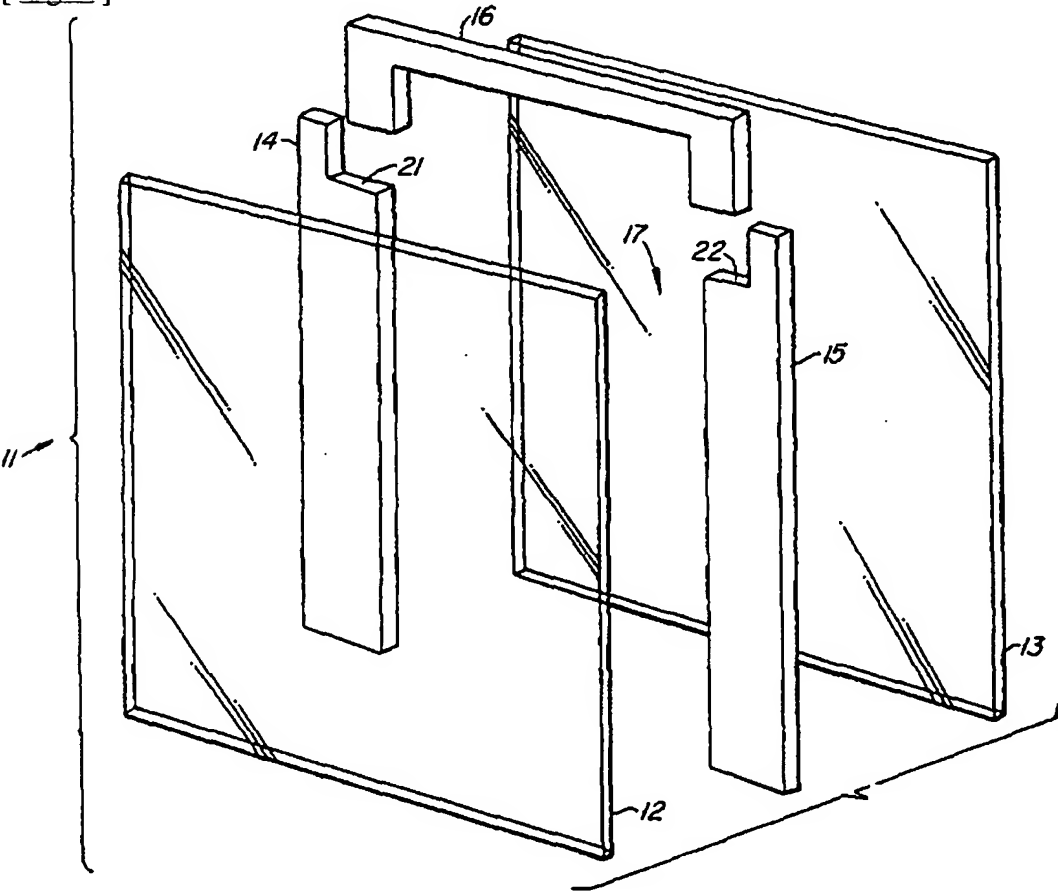
* NOTICES *

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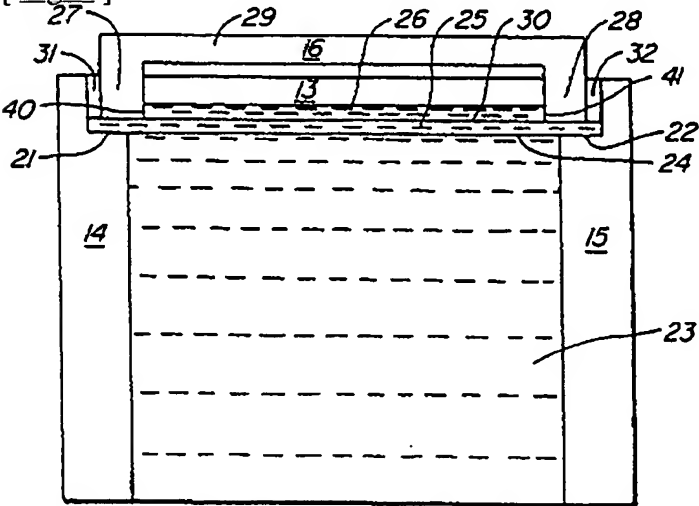
- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DRAWINGS

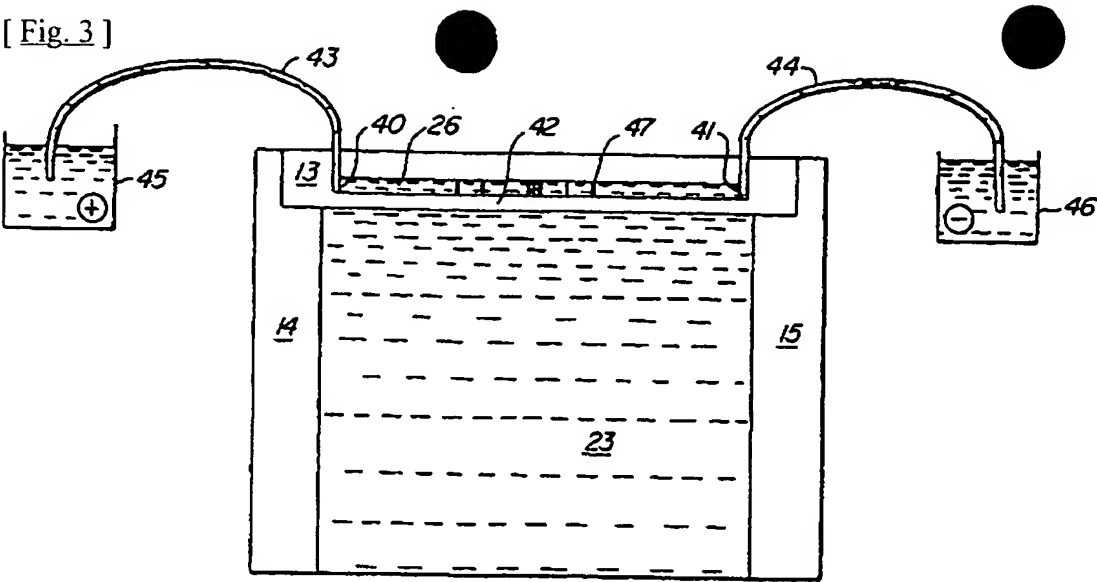
[Fig. 1]



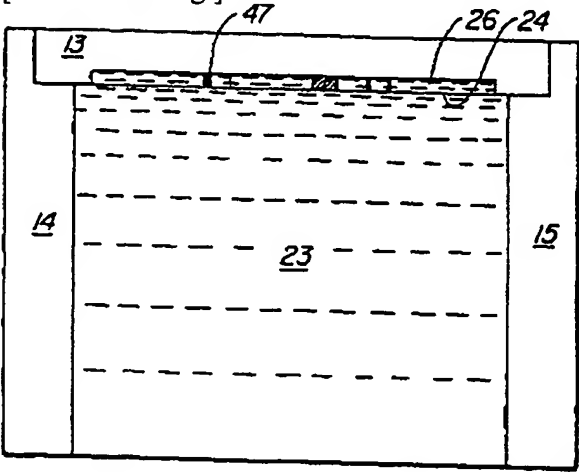
[Fig. 2]



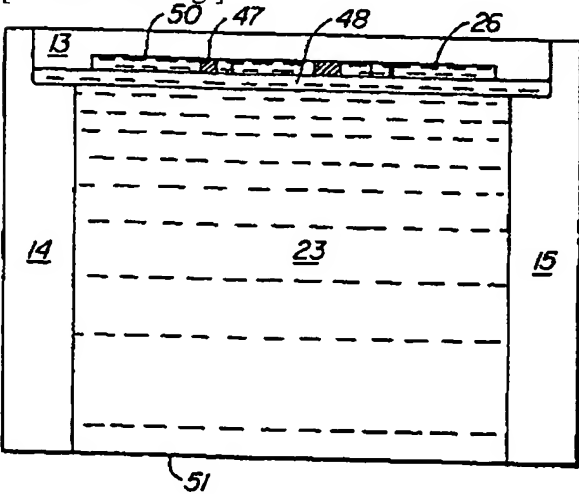
[Fig. 3]



[-- the -- 4a Fig.]



[-- the -- 4b Fig.]



[Translation done.]